IgE-mediated sensitization to malassezia in atopic dermatitis: More common in male patients and in head and neck type

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Abstract: BACKGROUND: Atopic dermatitis (AD) is a common chronic inflammatory skin disease. Malassezia, the predominant skin microbiota fungus, is considered to exacerbate AD, especially in a subset of patients with head and neck type AD (HNAD). In the present study, the relationship between AD and sensitization to Malassezia antigens was investigated. METHODS: We assessed 173 patients with AD. The severity of eczema was determined with Eczema Area and Severity Index (EASI); the type of AD, namely, head and neck type, was reported as well. The total serum IgE and specific IgE to Malassezia were determined and correlated with clinical picture of AD, sex, age, and the EASI. RESULTS: Total IgE was elevated in 77.7% of patients. Specific IgE to Malassezia was positive (0.35 kU/L) in 49.1% of patients. Men were significantly more often sensitized to Malassezia antigen (58% of men vs 42% of women; P value, 0.04). Concurrently, 58% of patients with HNAD versus 42% non-HNAD patients had higher levels of specific IgE to Malassezia, this difference being nearly significant (P value, 0.06). Patients with atopy were also more frequently sensitized to Malassezia. No significant relationship between EASI and the level of total IgE or specific IgE to Malassezia was observed. CONCLUSIONS: In our population, IgE-mediated sensitization was found in up to 49% of all patients with AD, most common in men and in head and neck type.

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IgE-Mediated Sensitization to *Malassezia* in Atopic Dermatitis: More Common in Male Patients and in Head and Neck Type

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Background: **Atopic dermatitis (AD) is a common chronic inflammatory skin disease. *Malassezia*, the predominant skin microbiota fungus, is considered to exacerbate AD, especially in a subset of patients with head and neck type AD (HNAD). In the present study, the relationship between AD and sensitization to *Malassezia* antigens was investigated.**

Methods: **We assessed 173 patients with AD. The severity of eczema was determined with Eczema Area and Severity Index (EASI); the type of AD, namely, head and neck type, was reported as well. The total serum IgE and specific IgE to *Malassezia* were determined and correlated with clinical picture of AD, sex, age, and the EASI.**

Results: **Total IgE was elevated in 77.7% of patients. Specific IgE to *Malassezia* was positive (≥0.35 kU/L) in 49.1% of patients. Men were significantly more often sensitized to *Malassezia* antigen (58% of men vs 42% of women; *P* value, 0.04). Concurrently, 58% of patients with HNAD versus 42% non-HNAD patients had higher levels of specific IgE to *Malassezia*, this difference being nearly significant (*P* value, 0.06). Patients with atopy were also more frequently sensitized to *Malassezia*. No significant relationship between EASI and the level of total IgE or specific IgE to *Malassezia* was observed.**

Conclusions: **In our population, IgE-mediated sensitization was found in up to 49% of all patients with AD, most common in men and in head and neck type.**

Atopic dermatitis (AD) is a common chronic inflammatory skin disease that exhibits repeated periods of remission and deterioration.[1] It affects 10% to 20% of children and 1% to 3% of adults in industrialized countries and its prevalence still rises. Atopic dermatitis usually begins within the first 2 years of life. Numerous IgE-inducing allergens play a role in the pathogenesis of AD. In fact, specific IgE antibodies against environmental allergens such as mites and various food allergens are detectable in sera of patients with AD.[1] Most patients with AD develop IgE sensitizations to various allergens during the course of the disease. The earliest sensitization to food allergens in children with AD occurs already in the first years of life, whereas sensitization to aeroallergens develops later, which is parallel with the progress of the atopic march.[2–4] The opportunistic yeast *Malassezia* species (formerly known as *Pityrosporum*) belongs to the normal cutaneous flora. *Malassezia* can also cause IgE-mediated sensitization in patients experiencing AD. Sensitization to microbial allergens seems to occur later in the course of AD than sensitization to food allergens and aeroallergens.[5] The role of the specific IgE in the pathogenesis of AD and its value as a marker for the severity of eczema are not completely understood.[5]

The impaired epidermal barrier and the altered immune system facilitate the penetration of allergens through the skin and their subsequent recognition by receptors. This results in enhanced release of IL4, IL5, and IL13 and increased production of IgE.[6] As a result, 50% of children[7] and 80% of adults[8] with AD have elevated levels of total serum IgE. Some studies show a statistical correlation between total IgE levels and the severity of eczema.[9–12] However, 20% of patients with manifest AD have never exhibited elevated serum levels of total or specific IgE,[13] the eczema in patients with very high IgE levels does not necessarily improve after the treatment with anti-IgE antibodies,[14] and the total IgE does not correlate with the severity of eczema in many patients.[15] Thus, the correlation between total or specific IgE and the severity of eczematous skin lesions in patients with AD is still controversial.

Approximately 50 fungal species colonize the skin of patients with AD,[16] the predominant fungus being the lipophilic yeast *Malassezia*. Concurrently, among the group of fungal allergens, sensitization to *Malassezia* species seems to be the most important, when compared with the relatively low sensitization rates for *Candida albicans* and *Cladosporium herbarum*.[5] *Malassezia* colonizes

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sebum-rich areas such as head, face, and neck rather than limbs or trunk because this microorganism requires human skin lipids in sebum for growth. The sebum production is high in the first 3 months of life, then decreases in childhood and again increases during puberty until the age of 50 years.[17] Therefore, age plays an important part in skin colonization with *Malassezia* species.

Atopic dermatitis of the head and neck (HNAD) is recognized as a separate condition. Patients with HNAD are more likely to have positive skin-prick test (SPT) results and *Malassezia*-specific IgE compared with healthy control subjects and patients with atopy without HNAD.[18] The reaction to *Malassezia* is likely related to both humoral- and cell-mediated immunities.[18] Clinically, *Malassezia* allergy may be suspected in patients with AD and in (1) head and neck lesions, (2) exacerbations during adolescence or young adulthood caused by increased sebaceous gland activity supporting *Malassezia* colonization, (3) severe lesions recalcitrant to conventional therapy, and (4) other atopic diseases.[18] There are some reports suggesting that these patients will benefit from antimycotic therapy.[18–20]

PATIENTS AND METHODS

Patients

We assessed 173 patients (96 women and 77 men) with AD seen in years 2011 and 2012 at the Department of Dermatology, University Hospital in Zürich. The only inclusion criterion was the presence of AD. The diagnosis of AD was confirmed by a dermatologist in each patient according to the clinical criteria of Hanifin and Rajka. The subgroup of patients with AD with primarily head, neck, and upper torso pattern of dermatitis was considered as patients with HNAD. The severity of eczema was assessed by a dermatologist using the Eczema Area and Severity Index (EASI) during a random visit of the patient at the Department of Dermatology in Zürich. The intensity of erythema, thickness (induration, papulation, edema), excoriation, lichenification, and the approximate percentage affected by eczema were calculated for each region.

Testing of IgE Antibodies

Serum sample was drawn from each patient for determination of total serum IgE and specific IgE. Specific IgE against *Malassezia* mix (m227), a mixture of 3 *Malassezia* species (*Malassezia sympodialis, Malassezia globosa,* and *Malassezia restricta*), was analyzed in all patients. When atopy was suspected (in 143 patients), specific IgE values against airborne and food allergens were also analyzed. Allergen-specific values of greater than or equal to 0.35 kU/L were regarded as positive. The samples of 133 patients were also analyzed for the presence of total IgE antibodies using the ImmunoCAP, a fluorescence enzyme immunosorbent assay (Thermo Fisher Scientific, formerly Phadia). Total serum IgE of greater than or equal to 100 kU/L was considered as elevated. The assessor was blinded to the outcome of the IgE results at the moment of classification into the HNAD or the non-HNAD group.

TABLE 1. Clinical Characteristics of All Patients in Regard of Sex and Head/Neck Type

	All	Female	Male	HNAD	Non-HNAD
Patients total	173	55.5% (96/173)	44.5% (77/173)	42.8% (74/173)	57.2% (99/173)
Mean age, y	37.6	37.3	38.3	37.5	37.6
Age groups, y					
4–25	28.9% (50/173)	28.1% (27/96)	29.9% (23/77)	27% (20/74)	30.3% (30/99)
25–84	71.1% (123/173)	71.9% (69/96)	70.1% (54/77)	73% (54/74)	69.7% (69/99)
Median total IgE, kU/L*	407	255	1131	637	295
		P value: <0.001		*P* value: 0.06	
Elevated total IgE (≥100 kU/L)*	77.7% (94/133)	62.5% (45/72)	80.3% (49/61)	82.4% (42/51)	63.4% (52/82)
		P value: 0.04		*P* value: 0.69	
Median sp IgE *Malassezia*, kU/L	0.25	0.1	0.57	1.3	0.13
		P value: <0.001		*P* value: <0.001	
Positive sp IgE *Malassezia* (≥0.35 kU/L)	49.1% (85/173)	41.7% (40/96)	58.4% (45/77)	58.1% (43/74)	42.4% (42/99)
		P value: 0.04		*P* value: 0.06	
Atopy† (positive sp IgE to airborne or food allergens)	68.5% (98/143)	59% (46/78)	80% (52/65)	71.2% (42/59)	66.7% (56/84)
		P value: 0.01		*P* value: 0.7	
HNAD	42.8% (74/173)	35.4% (34/96)	51.9% (40/77)		
		P value: 0.04			
Sex					
Female				46% (34/74)	62.6% (62/99)
Male				54% (40/74)	37.4% (37/99)

A P value of less than 0.05 was considered to be significant.
HNAD indicates head and neck atopic dermatitis; sp IgE, specific IgE; y, years.
**Total IgE (≥100 kU/L) was not evaluated in 40 patients (24 women and 16 men; 23 HNAD and 17 non-HNAD).*
†Atopy was not evaluated in 30 patients (18 women and 12 men; 15 HNAD and 15 non-HNAD).

Statistical Analysis

Continuous data of nonparametric distribution are presented as median values; the variability of the data was characterized by minimum-maximum intervals and first and third quartile range. Differences between groups were calculated by means of Mann-Whitney-Wilcoxon test. Differences in proportions between groups were tested by Pearson χ^2 test. *P* values of less than or equal to 0.05 were considered statistically significant.

RESULTS

The clinical characteristics of patients are summarized in Tables 1 and 2.

We assessed 173 patients with AD (96 women and 77 men). Median total serum IgE was 407 kU/L. Elevated total IgE (≥100 kU/L) was detected in 77.7% of patients. Specific IgE to *Malassezia* was detected in 49.1% of patients. Atopy (positivity of specific IgE to airborne or food allergens) was found in 68.5% of patients. Men were significantly more often sensitized to *Malassezia* antigens and had significantly higher levels of specific IgE to *Malassezia* than women (Fig. 1). Concurrently, men had significantly more often elevated total IgE (≥100 kU/L) and also had higher levels of total IgE (Fig. 2). We found atopy significantly more often in men than in women. Men also experienced HNAD significantly more often than women.

We divided the patients into 2 groups, according to clinical picture of AD. Seventy-four (42.8%) patients experienced HNAD with predominant involvement of the face, scalp, and upper torso. Ninety-nine (57.2%) patients had a more generalized form of AD or dermatitis on hands or feet, which does not involve the head and especially the neck area. In the non-HNAD group, minimal eczematous lesions in small areas with typical AD locations (eg, eyelid, retroauricular) at some time points were present; however, no neck involvement at all and no major involvement of the face (<10% of the surface of the face) was allowed in this group.

Patients with HNAD had significantly higher levels of *Malassezia*-specific IgE (Fig. 3). We observed only a trend to more frequent sensitization to *Malassezia* antigens and to higher levels of total IgE in patients with HNAD (Fig. 4). Atopy occurs with approximately equal frequency in both groups (HNAD and non-HNAD). We divided the patients into 2 age groups. Fifty patients belonged in the group of children and young adults (ages, 4–25 years; 9 prepubertal children were 12 years or younger) and 123 patients were older than 25 years. Atopy was significantly more often present in children and young adults. We observed no other significant differences in these 2 age groups.

Positive specific IgE to airborne or food allergens was found in 68.5% of patients. In these patients, we observed significantly higher levels and more frequent positive specific IgE to *Malassezia* antigens (Fig. 5). There was no difference in frequency of atopy between patients with HNAD and patients with skin lesions in other localizations.

TABLE 2. Clinical Characteristics and Sensitization Patterns According to Age and Atopy

	0–25 y	>25 y	Atopy	Without Atopy
Patients total	28.9% (50/173)	71.1% (123/173)	68.5% (98/143)	31.5% (45/143)
Mean age, y	18.6	45.5	37.2	41.4
Sex				
Female	54% (27/50)	56.1% (69/123)	46.9% (46/98)	71.1% (32/45)
Male	46% (23/50)	43.9% (54/123)	53.1% (52/98)	28.9% (13/45)
Median total IgE, kU/L*	368	453	1226	44.35
	P value: 0.39			
Elevated total IgE (≥100 kU/L)*	64.7% (22/34)	72.7% (72/99)	76.5% (75/98)	22.2% (10/45)
	P value: 0.12			
Median sp IgE *Malassezia*, kU/L	0.64	0.23	1.52	0
	P value: 0.26		*P* value: <0.001	
Positive sp IgE *Malassezia* (≥0.35 kU/L)	56% (28/50)	46.3% (57/123)	68.4% (67/98)	11.1% (5/45)
	P value: 0.33		*P* value: <0.001	
Atopy† (positive sp IgE to airborne or food allergens)	84.6% (33/39)	62.5% (65/104)		
	P value: 0.02			
HNAD	40% (20/50)	43.9% (54/123)	42.9% (42/98)	37.8% (17/45)
	P value: 0.76			
Age groups, y				
0–25			33.7% (33/98)	13.3% (6/45)
25–84			66.3% (65/98)	86.7% (39/45)

A P value of less than 0.05 was considered to be significant.
HNAD indicates head and neck atopic dermatitis; sp IgE, specific IgE; y, years.
**Total IgE (≥100 kU/L) was not evaluated in 40 patients (0–25 years not applicable in 16 patients, and 25–84 years not applicable in 24 patients).*
†Atopy was not evaluated in 30 patients (0–25 years not applicable in 11 patients and 25–84 years not applicable in 19 patients; 18 women and 12 men; 15 HNAD and 15 non-HNAD).

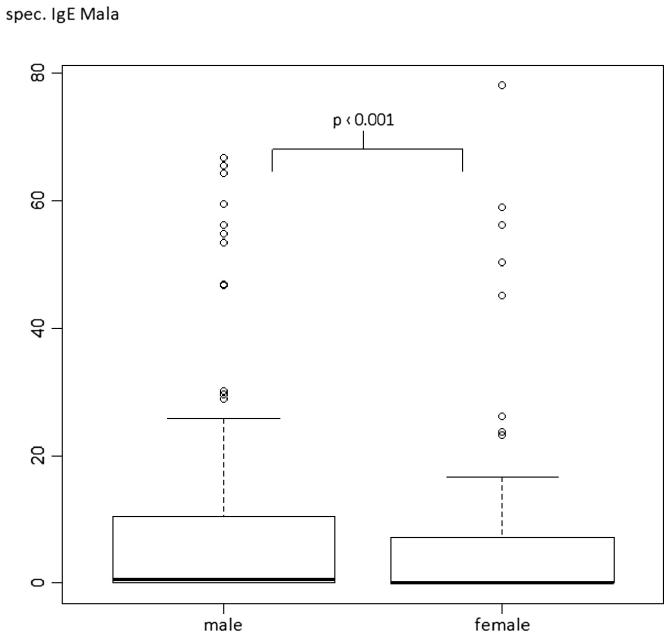

Figure 1. Comparison of specific IgE against *Malassezia* (Mala) in relation to sex.

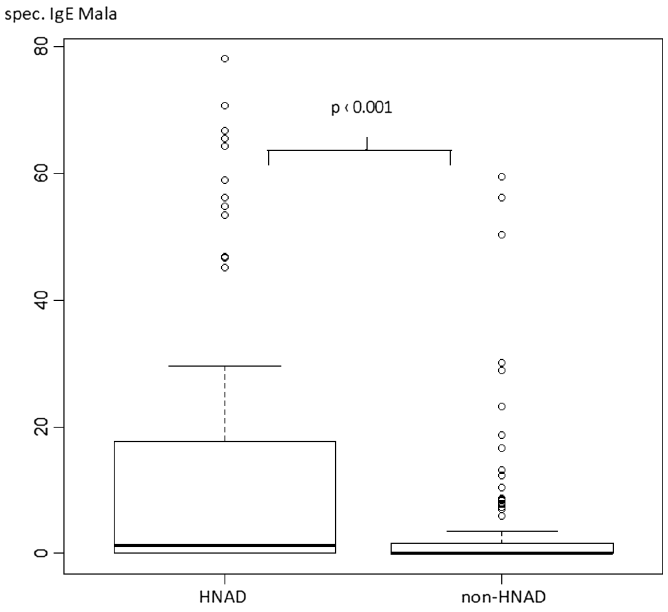

Figure 3. Comparison of specific IgE against *Malassezia* (Mala) in relation to head and neck type.

Eczema Area and Severity Index was evaluated only in 32 patients, and the mean EASI was 23.1. Most patients were diagnosed with mild to moderate eczema. We did not find significant relationship between EASI and specific IgE to *Malassezia* or levels of total IgE.

DISCUSSION

Barrier function defect is a result of altered filaggrin, increased pH, decreased antimicrobial secretions, decreased IgA, and decreased sweat production.[21] The alkaline pH may stimulate the release of antigens from *Malassezia*, and the decreased antimicrobial peptides may allow *Malassezia* to grow.[21] In response to dense *Malassezia* colonization, keratinocytes produce T-helper 2 cytokines and chemokines and the local antigen-presenting cells (APCs) are activated. The activated APCs take up *Malassezia* antigens and present them to T cells. T cell–APC interactions result in T-cell activation and production of additional T-helper 2 cytokines and chemokines and

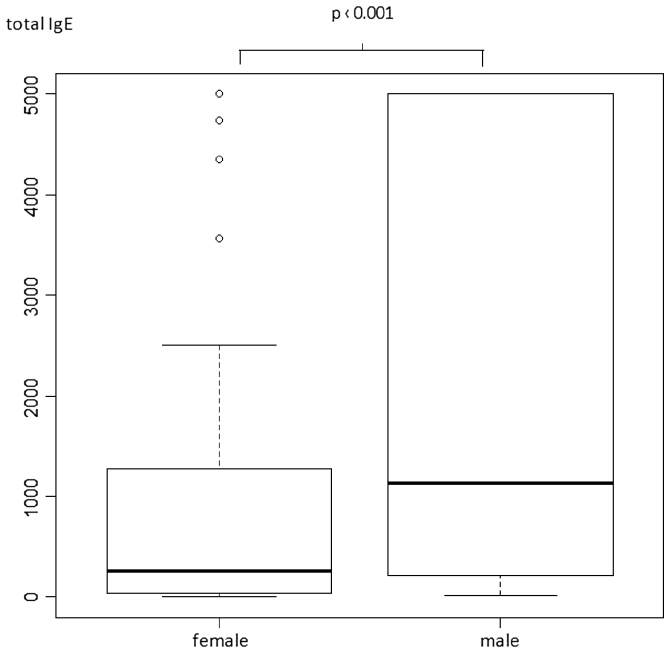

Figure 2. Comparison of total IgE in relation to sex.

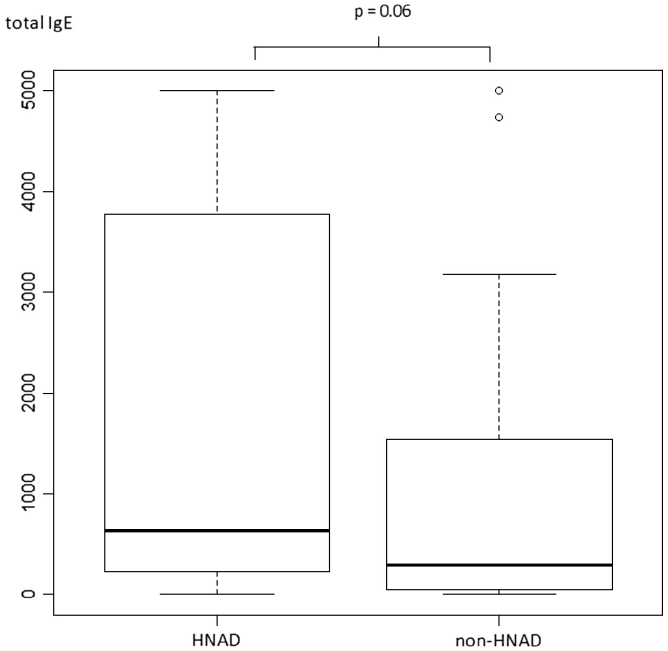

Figure 4. Comparison of total IgE in relation to head and neck type.

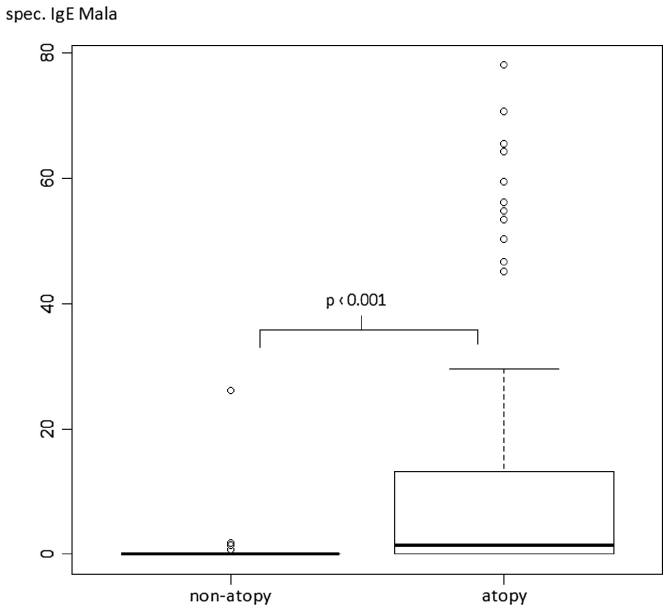

Figure 5. Comparison of specific IgE against *Malassezia* (Mala) in relation to atopy or nonatopy.

up-regulation of adhesion molecules. This may lead to influx of more leukocytes into *Malassezia*-colonized skin, resulting in increased inflammation in the densely *Malassezia*-colonized areas causing the clinical entity of HNAD.[18] During this process, B cells are also stimulated by APCs and T-helper 2 cytokines. This results in isotype switching and production of *Malassezia*-specific IgE. Table 3 summarizes the possible effects and interaction of *Malassezia* and worsening of skin barrier.

Given the widely accepted multifactorial cause of AD, it is unlikely that immunologic reactions against *Malassezia* would be a single causative factor of HNAD. However, it could become a triggering factor. There is no convincing evidence that the skin of patients with AD is more heavily colonized with *Malassezia* or is colonized with different species of *Malassezia*, when compared with healthy control subjects or patients with seborrheic dermatitis. Many studies have tried to find a different pattern of *Malassezia* colonization of AD skin, which is not present on healthy skin.[22–27] However, the published results are controversial.[18] The challenges of proper sampling, in vitro culture, and correct identification of *Malassezia* species could be the reason for conflicting results. Nevertheless, compared with healthy control subjects, patients with atopy are more likely to develop an immune response after exposure to any antigen.[18] Several studies have shown that patients with AD react more frequently to *Malassezia* antigens on SPTs and exhibit higher levels of *Malassezia*-specific IgE compared with healthy control subjects.[28–31] Focusing on the subset of patients with HNAD, research has shown that these patients tend to have higher rates of positive SPT results and higher levels of *Malassezia*-specific IgE when compared with patients who have AD without HNAD.[29,32–34] This corresponds also to our results. One of the larger studies enrolled 589 patients and found that 100% of patients with HNAD had increased *Malassezia*-specific IgE compared with only 13% of patients with AD without HNAD.[35] Our results are not so convincing. In our investigation, 58.1% of patients with HNAD and 42.4% of patients with AD without HNAD had increased *Malassezia*-specific IgE. Two former studies have even reported a correlation between *Malassezia*-specific IgE levels and the clinical severity of HNAD.[34,35] In contrast to these results, we could not confirm that higher total IgE or specific IgE to *Malassezia* would serve as markers for more severe eczema. We did not find a significant relationship between EASI and specific IgE to *Malassezia*. This may be due to the limited number of patients with EASI scoring involved in our study. Nevertheless, we assume that the more severe disease leads to higher likelihood of sensitization. Studies of SPT and specific IgE suggest that the immune system of patients with AD recognizes *Malassezia* antigens and induces a B-cell–mediated humoral response against these antigens.[18] In contrast, the atopy patch test (APT) assesses the T-cell–mediated response to an antigen. In any case, T-cell mediation is critical to dermatitis, which is a delayed-type hypersensitivity response, but also by leading to T_H2 responses favoring sensitization in AD.[36] The use of the APT in *Malassezia*-mediated sensitization in AD is somewhat controversial. Some studies suggest that APT is more specific than tests for antigen-specific IgE.[37] In another study, APT was a less sensitive test for demonstrating *Malassezia*-specific IgE-mediated immune response.[18] However, in other studies, no

TABLE 3. Possible Effects of *Malassezia* Colonization and Skin Function in AD

	Skin Function in AD	*Malassezia* Colonization
Skin barrier	Impaired by various factors such as filaggrin deficiency Allows presentation of *Malassezia* to immune system	Activation of mast cell IgE responses leading to increased skin inflammation with possible association with[48] worsening of skin barrier function
pH	Alkaline pH stimulates growth of *Malassezia* and increased expression of allergens.[21]	
Effect on keratinocytes		Production of T-helper 2 cytokines Activation of APCs Up-regulation of chemokines and adhesion molecules

AD indicates atopic dermatitis; APCs, antigen-presenting cells.

correlation between tests for antigen-specific IgE and APT was found.[38] It is possible that systemic and topical antiyeast therapies would be most effective in patients with cutaneous hypersensitivity to *Malassezia* as demonstrated in positive APT.[38] Thus, also, APT in *Malassezia* sensitizations might have a relevant role in the workup of AD. Some of the variability seen in the results of different studies may be related to the different methods of *Malassezia* culture, conditions, extract storage, and selection of antigens. The stability of *Malassezia* extracts has been reported to be poor, even when stored at +4°C, and it is recommended that storage should not exceed more than 1 month.[39] It is also recommended that culture extracts containing the highest number of antigenic proteins should be used in clinical testing to avoid false-negative results. In our study, *Malassezia* mix (m227) representing 3 different species was used. This allowed the detection of more *Malassezia*-sensitized individuals than does the analysis of specific IgE to *M. sympodialis* (m70) only.

Recent literature describes elevated total IgE in roughly 80% of adults and 50% children with AD.[7,8] Concerning the fungal allergens tested in our investigation, previous studies found sensitization to *Malassezia* species in 12% to 17% of children[40–42] and in 29% to 65% of adults.[31,43] The considerably higher rate of sensitization in adults than in children described in previous studies was probably due to the longer disease history with a higher time-dependent probability to develop multiple sensitizations in adults.[10] In contrast, in our study, we did not find significant correlation between age and total IgE or sensitization to *Malassezia* species. We observed only a trend to more frequent elevated total IgE in older patients, surprisingly with an opposite trend concerning specific IgE to *Malassezia*.

CONCLUSIONS

In our study, nearly half of patients with AD were sensitized to *Malassezia* antigen. We found that men and patients with HNAD were more frequently sensitized to *Malassezia* antigens and also had significantly higher levels of specific IgE to *Malassezia*. Sebaceous gland activity is increased in men and in sebum-rich areas such as head, face, and neck, so our results support the seborrhea-dependent theory of *Malassezia* sensitization. Obviously, IgE-mediated sensitization against *Malassezia* is not the single causative factor of AD, but it could act as a triggering factor. However, there is a close interaction between the presence of *Malassezia* and the factors influencing skin in AD such as pH, the barrier function, or biofilm composition.[44,45] The reduction or elimination of triggering microbial factors on the skin, for example, by using antifungals,[46,47] in combination with anti-inflammatory and emollient therapy, may decrease the immune and inflammatory response and considerably improve the clinical course of AD.

Recent studies have involved populations from other regions (eg, Scandinavia,[25] United States of America,[5] or Asian populations[1]). Our study confirms the findings of previous studies but also describes for the first time a more central European (Swiss) population including the aspects of HNAD/non-HNAD.

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